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Microbial Degradation of Trichloroethylene in the Rhizosphere: Potential Application to Biological Remediation of Waste Sites†

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The possibility that vegetation may be used to actively promote microbial restoration of chemically contaminated soils was tested by using rhizosphere and nonvegetated soils collected from a trichloroethylene (TCE)-contaminated field site. Biomass determinations, disappearance of TCE from the headspace of spiked soil slurries, and mineralization of [^{14}C]TCE to $^{14}CO_2$ all showed that microbial activity is greater in rhizosphere soils and that TCE degradation occurs faster in the rhizosphere than in the edaphosphere. Thus, vegetation may be an important variable in the biological restoration of surface and near-surface soils.

Plant roots provide a microhabitat conducive to the proliferation of soil microorganisms. Sloughing of epidermal and cortical cells, release of enzymes, and exudation of nutrients such as amino acids, simple sugars, and complex carbohydrates are mechanisms by which soils are enriched by roots and a more favorable microhabitat is created for microbial life (2). Similarly, microorganisms can increase the availability of nutrients to plants, decrease the vulnerability of roots to pathogens, synthesize and release growth factors, and decompose dead plant matter. The root-soil-microbe interaction is complex and appears to have evolved to the mutual benefit of many plant and microbial species.

Rhizosphere studies have yielded many applications for improved crop production and control of plant pathogens. In addition to these applications, we propose that the unique environment of the plant rhizosphere-rhizoplane may also function to enhance microbial degradation of hazardous organic compounds in soils. Support for the potential of vegetation to play a role in waste site restoration comes from studies of agricultural chemicals in which the persistence of several insecticides and herbicides in soils has been shown to decrease in the rhizosphere (5, 6, 13, 14). These losses appeared to be microbially mediated. Although this phenomenon is generally regarded as contrary to effective crop protection with agricultural chemicals, from our perspective these studies show promise that the unique environment of the root zone may be especially conducive to microbial degradation of persistent and hazardous compounds in surface and near-surface soils.

We collected soil samples from a former chlorinated-solvent disposal site and compared microbial degradation of trichloroethylene (TCE) in rhizosphere soils and nonvegetated (edaphosphere) soils. Findings from these experiments, which are presented below, provide strong evidence that vegetation may be used to actively promote microbial restoration of chemically contaminated surface and near-surface soils.

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MATERIALS AND METHODS

Site description. The Miscellaneous Chemicals Basin (MCB) at the U.S. Department of Energy's Savannah River Site, Aiken County, S.C., was used as a chemical disposal site beginning ca. 1956 (12). Originally, the MCB was a small, shallow basin (approximately 6 by 6 by 0.3 m) where waste chemicals were poured directly onto the soil. Fill was added when chemical disposal stopped (probably in 1974), and the site was graded to an area of approximately 100 by 100 m. The site became naturally vegetated with weeds, grasses, and small pine trees (≤10 years old).

Analyses of soil cores at the site showed residues of TCE, tetrachloroethylene, *trans*-1,2-dichloroethylene, and trichloromethane at the surface (12; T. C. Hazen, Savannah River Site, personal communication). Sediment cores to a depth of 100 m and subsequent monitoring wells revealed that the contaminants had not reached the water table and were confined to the upper 10 m of the vadose zone. A risk assessment of the MCB revealed no immediate threat to human health or the environment (12). Thus, several characteristics of the site made it a favorable location for exploring the potential for vegetation to be managed as an integral part of site remediation. Specifically, the area is small and inaccessible to the public, TCE and other hazardous chemicals are present in relatively low concentrations, and no imminent hazard exists. Moreover, the MCB has become vegetated naturally during the 10 or more years since chemical disposal stopped; thus, sufficient time has passed for the populations or consortia of TCE-degrading microorganisms to be enriched in the rhizosphere.

Soil and vegetation. Local experts were consulted to characterize the soil and identify the plant species at the MCB. Pedon descriptions were made as deep as 200 cm at nine sites within the MCB. This survey revealed that the area is mostly Udorthents firm substratum, and the basin resulted from removal of much of the developed surface soil. The remaining material showed low permeability and slow infiltration, a condition that produces a perched water table within ~0.3 to ~0.9 m of the surface during rainy seasons.

Plant species and relative abundance were described by using a grid system at the site. The predominant herbaceous species in this well-vegetated area were a grass, *Paspalum notatum* var. *saurae* Parodi; a legume, *Lespedeza cuneata*

(Dumont); and a composite herb, a *Solidago* sp. Loblolly pine (*Pinus taeda* L.) was also present. All of these species are plentiful in the area surrounding the MCB.

Soil samples were taken from the root zones of the four predominant plant species at the MCB, from nonvegetated areas with TCE contamination, and from nonvegetated areas where TCE was not present. (Chemical analyses subsequent to sampling confirmed that all soil samples were contaminated with TCE, with the exception of the nonvegetated, noncontaminated sample. The latter had no detectable TCE; however, trace dichloroethylene was detectable.) The nonvegetated areas were patchily distributed within and around the MCB and were devoid of roots. Herbaceous species were uprooted, and soil was tapped from the roots onto clean plastic sheets. Soils were collected from pine trees by scraping aside the top 1 to 2 cm and then using a trowel to remove soil adhering to and immediately surrounding the roots. Nonvegetated soils were sampled in a similar manner. Soils of each type were mixed to produce composite samples, transferred to sterile Whirl Pak bags (NASCO, Fort Wilkinson, Wis.), sealed, and stored on ice for transport to Oak Ridge National Laboratory. The soils, which were finely sieved (2.0 mm) before use, were stored in the dark at 4°C.

The pH and percent organic carbon (% OC) of composite soil samples were determined at Oak Ridge National Laboratory. Carbon analyses were done in triplicate by using a WR12 Carbon Determinator (Leco, St. Joseph, Mich.) equipped with an HF20 induction furnace (9). Inorganic carbon sources were insignificant in this soil; therefore, total carbon represents organic carbon. Soil pH measurements (8) were made in distilled water at a soil-to-water ratio of 1 g:2 ml.

Microbial respiration. Carbon dioxide efflux was measured from rhizosphere soils for four plant species and the two edaphosphere soils (TCE-contaminated and noncontaminated) to compare respiration among soil sample types and to estimate microbial biomass. Triplicate soil samples (50 g) were moistened to 80% saturation with distilled, deionized water and incubated in the dark at 20°C in 8- by 5-cm glass jars. An infrared gas analyzer (LIRA Model 3000, Mine Safety Appliances Company, Pittsburgh, Pa.) was used to monitor CO_2 efflux (3, 16) at 24-h intervals for 7 days. Carbon dioxide respiration was used to calculate microbial biomass by the method of Jenkinson and Powlson as modified by Anderson and Domsch (11).

Headspace analysis. Initial experiments to monitor the disappearance of TCE from the headspaces of soil slurries were undertaken to provide an indication of whether biological degradation of TCE occurred in soil samples. Duplicate soil samples (3 g) from each of the four plant species, as well as from nonvegetated areas of the MCB, were placed in 40-ml glass bottles equipped with screw caps and polytetrafluoroethylene-lined septa. Distilled, deionized water (20 ml) spiked with TCE at either 35 or 70 μg/ml was added. Sterile controls for each soil sample type and sterile water controls (no soil) were prepared by autoclaving samples for 1 h on each of three consecutive days before use. Samples were incubated in the dark at 20°C on a shaker table (125 rpm).

Headspace concentrations of TCE were determined with a Sigma 2000 capillary gas chromatograph (The Perkin-Elmer Corp., Norwalk, Conn.) equipped with an electron capture detector. Chromatographic conditions were as follows: column, SP-1000 (0.32 cm [inside diameter] by 61 cm); carrier gas, N_2 (30 ml/min); injector temperature, 100°C; column temperature, 100°C; detector temperature, 350°C. Concentrations of TCE were quantified by integration of peak areas. The coefficient of variation ([standard deviation × 100]/mean) for measurements of TCE in the headspace of the distilled, deionized water was 7.0%.

TABLE 1. % OC and pH of study soils from the MCB at the Savannah River Site

Soil source[a]	% OC ± SD (n = 3)	pH ± SD (n = 3)
Nonvegetated, noncontaminated	0.20 ± 0.04[b]	5.20 ± 0.10[b]
Nonvegetated	1.06 ± 0.08[b,c]	6.39 ± 0.09[b]
L. cuneata	1.35 ± 0.01[b,d]	6.81 ± 0.01[e]
Solidago sp.	1.20 ± 0.05[d]	5.53 ± 0.15[b]
P. notatum var. *saurae*	1.43 ± 0.05[b,d]	6.75 ± 0.05[e]
P. taeda	1.26 ± 0.03[d]	6.87 ± 0.09[e]

[a] All soil samples were from MCB areas contaminated with TCE with the exception of the nonvegetated, noncontaminated sample. The latter had no detectable TCE; however, trace dichloroethylene was detectable.
[b] Significantly different from all other soils ($P \leq 0.05$).
[c] Significantly greater than nonvegetated, noncontaminated soil ($P \leq 0.05$).
[d] Significantly greater than nonvegetated soil and nonvegetated, noncontaminated soil ($P \leq 0.05$).
[e] Significantly different from the *Solidago* sp., nonvegetated soil, and nonvegetated, noncontaminated soil ($P \leq 0.05$).

Mineralization of [^{14}C]TCE. The role of microorganisms in the biodegradation of TCE was further explored by comparing mineralization of [^{14}C]TCE in soil from the root zone of one plant species (*L. cuneata*) with that of the nonvegetated TCE-contaminated soil. Triplicate soil samples (50 g) for both treatment groups plus sterile controls (autoclaved for 1 h on three consecutive days) were moistened with sterile, distilled, deionized water to 80% saturation. The [^{14}C]TCE (specific activity, 1.12 × 10^6 Bq/mmol; chemical purity, >99%; Sigma Chemical Co., St. Louis, Mo.) was added to the soil at 70 μg/g of soil (dry weight) in glass sample jars (8 by 5 cm) and closed with polytetrafluoroethylene-lined neoprene stoppers.

At 24-h intervals, filtered air was used to flush the sample jars and then was passed sequentially through 7 ml of ethylene glycol monomethyl ether (Fisher, Fairlawn, N.J.) and 10 ml of $^{14}CO_2$ UNT SORB cocktail (Research Products International, Elk Grove, Ill.) to trap ^{14}C-labeled volatiles and $^{14}CO_2$, respectively (15). Samples (200 μl) of the ethylene glycol monomethyl ether were counted in Aqueous Counting Scintillant (Amersham, Arlington Heights, Ill.) on a liquid scintillation spectrometer (Tri-Carb 2000 CA; Packard Instrument Co., Inc., Rockville, Md.). The $^{14}CO_2$ cocktail was counted directly.

Statistical analyses. The significances of differences among samples for measurements of pH, organic carbon, biomass, and mineralization of [^{14}C]TCE were evaluated by using Student's t test ($P \leq 0.05$).

RESULTS

Soil and vegetation surveys. The soil samples proved to be low in organic carbon and slightly acidic (Table 1), which is typical of areas where rainfall is abundant and warm, humid conditions prevail. The % OC was lower ($P \leq 0.05$) for the nonvegetated soils (non-TCE contaminated, 0.20% OC; TCE contaminated, 1.06% OC) than for the rhizosphere soils, which ranged from 1.20 to 1.43% OC. With the exception of the *Solidago* sp., the rhizosphere soils had higher pHs than the nonvegetated soils. Differences in these parameters were expected and were consistent with the fact

TABLE 2. Carbon dioxide efflux from rhizosphere and edaphosphere soils collected at the MCB at the Savannah River Site[a]

Soil source[b]	Net CO_2 efflux[c] (μg/g of soil per h) ± SD (n = 3)	Biomass (μg/g of soil)[d]
Nonvegetated, noncontaminated	0.62 ± 0.04	283
Nonvegetated	0.83 ± 0.17	379
L. cuneata	4.93 ± 1.66[e,f]	2,253
Solidago sp.	5.90[f]	2,697
P. notatum var. *saurae*	4.17 ± 0.68[f]	1,906
P. taeda	3.52 ± 0.49[f]	1,609

[a] Data are for day 3 of incubation in the dark at 20°C.

[b] All soil samples were from MCB areas contaminated with TCE, with the exception of the nonvegetated, noncontaminated sample. The latter had no detectable TCE; however, trace dichloroethylene was detectable.

[c] Net CO_2 efflux = CO_2 efflux from soil sample (triplicate determinations) − CO_2 efflux from matched, autoclaved soil (triplicate determinations).

[d] Biomass was calculated by the methods of D. Parkinson and E. A. Paul (11).

[e] A matched sterile control was not available; therefore, net CO_2 efflux was calculated by subtracting efflux for the sterile control for *P. notatum*. The mean CO_2 efflux was not significantly different for the nonsterile *P. notatum* and *L. cuneata* soils.

[f] Significantly greater than CO_2 efflux from nonvegetated, contaminated soil and nonvegetated, noncontaminated soil ($P \leq 0.05$).

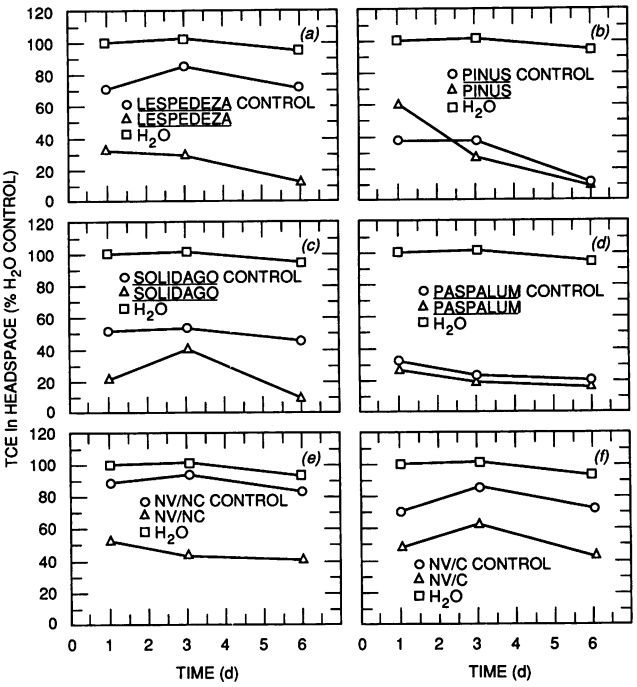

FIG. 1. Headspace concentrations of TCE in sterile, distilled water (□) and soil slurries prepared from rhizosphere and edaphosphere soils collected at the MCB, Aiken County, S.C. Headspace concentrations of TCE are shown for aqueous slurries prepared from (a) *L. cuneata*, (b) *P. taeda*, (c) the *Solidago* sp., (d) *P. notatum* var. *saurae*, (e) nonvegetated (NV), noncontaminated (NC) soil, and (f) nonvegetated (NV), contaminated (C) soil and are shown as the percentage of the headspace concentration above sterile distilled water. Each graph also shows TCE in the headspaces of slurries prepared from sterile soil of each type (control). Each datum point is the mean of at least two treatments. The coefficient of variation ([standard deviation × 100]/mean) for measurements of TCE in the headspace of the distilled, deionized water was 7.0%. Time is indicated in days (d).

that some soils received waste solvent input and were vegetated whereas others were not. Because the primary purpose of the experiments was to determine whether degradation of TCE was enhanced in the rhizosphere, without regard at this time for the specific factors controlling such an effect, no attempt was made to normalize differences in % OC or pH between soil samples.

Microbial degradation. Carbon dioxide efflux from incubated soils shows a pattern consistent with the enhanced microbial activity in the plant rhizosphere, in that higher respiration rates ($P \leq 0.05$) were observed for all soils from the rhizosphere than for soils from the edaphosphere. For example, on day 3, CO_2 efflux was from four to nine times greater in rhizosphere soils than in edaphosphere soils (Table 2). Consequently, the calculated biomass was consistently higher in rhizosphere soils than in edaphosphere soils (Table 2).

Analysis of the headspaces above aqueous soil slurries spiked with TCE showed that the solvent was lost from the headspaces of rhizosphere soils more quickly (Fig. 1a through d) than from edaphosphere soils (Fig. 1e and f) in all cases, as evidenced by TCE concentrations on the last sampling day. Throughout the 6-day sampling period, TCE concentrations were higher in the sterile water control (no soil) than in sterile soil slurries for all soils, indicating either nonbiological transformation of TCE or simply sorption of TCE to the soil. The fact that TCE concentrations decreased rapidly from day 0 to day 1 but changed only slightly from day 1 to day 6 in all sterile soil samples indicates that abiotic TCE losses occurred rapidly on day 1 of the experiment but contributed very little to TCE losses thereafter. This observation is consistent with sorption as the predominant abiotic loss process.

Biological transformation of the TCE was most evident as an important process in *L. cuneata* (Fig. 1a) and nonvegetated, noncontaminated soils (Fig. 1e), because the TCE loss from the soil compared with the matched sterile control was greatest for each of these samples. On day 6, there was 55% less TCE in the headspace of the *L. cuneata* soil compared with its matched sterile control (Fig. 1a), whereas the nonvegetated, noncontaminated soil showed 40% less TCE in the headspace compared with its matched sterile control (Fig. 1e) on day 6. These differences can be attributed directly to biological transformation of TCE in each soil type.

Differences in TCE concentrations were marked but less pronounced between sterile and nonsterile soil slurries of the *Solidago* sp. (Fig. 1c) and nonvegetated, contaminated soil (Fig. 1f). Once again, the faster disappearance of TCE from the nonautoclaved soils indicated that biological processes contribute to the disappearance of TCE. Rapid loss of TCE was also seen for *P. taeda* (Fig. 1b) and *P. notatum* (Fig. 1d); however, the contribution of microbial transformation could not be inferred because TCE was also lost from the headspaces of matched, sterile soils. Nonetheless, the rate of TCE disappearance from *P. taeda* and *P. notatum* exceeded that from nonvegetated soils (Fig. 1e and f). Thus, enhanced microbial degradation may still occur in these rhizosphere soils, but microbial degradation may not be distinguishable from other losses unless a higher TCE concentration is present.

The hypothesis that microbial degradation of TCE is faster in the rhizosphere than in the edaphosphere is also supported by the comparison of [^{14}C]TCE mineralization in *L.*

cuneata and nonvegetated, TCE-contaminated soils. Mineralization to $^{14}CO_2$ occurred in both soils; however, a significantly greater amount of $^{14}CO_2$ ($P \leq 0.05$) was produced in the rhizosphere soil from *L. cuneata* (mean ± standard deviation = 3.1 ± 0.42 ng of $^{14}CO_2$ per 50 g of soil over 30 days) than in the nonvegetated, TCE-contaminated soil (1.3 ± 0.68 ng of $^{14}CO_2$ per 50 g of soil). Moreover, $^{14}CO_2$ efflux from the latter soil was not significantly greater than that from its matched, sterile control or the sterile *L. cuneata* control.

DISCUSSION

Three different indices of microbial activity (biomass determinations, disappearance of TCE from the headspaces of spiked soil slurries, and mineralization of [^{14}C]TCE to $^{14}CO_2$) all provided evidence that microbial activity is greater in rhizosphere soils at the MCB and that TCE degradation occurs faster in the rhizosphere than in the edaphosphere. Because no living roots were present in the rhizosphere soils at the time of these experiments, the possibility for TCE losses because of sorption or uptake by plant roots was eliminated. However, a disadvantage of this experimental approach is that the absence of roots precluded the direct interaction of the roots with the microbial community in the soil. For this reason, the measurements of TCE loss from the rhizosphere soils and the observed rate of [^{14}C]TCE mineralization may be lower than that which actually occurs in the root zone of a living plant. Similarly, biodegradation of TCE may be much slower than biodegradation of less-persistent waste chemicals, such as nonhalogenated monoaromatics and diaromatics. Therefore, the data presented herein are likely to be a conservative indication of the potential for enhanced degradation of waste chemicals in the rhizosphere.

The data on TCE degradation in rhizosphere soils also provide a strong incentive to explore the soil-root-microbial interaction and the variables that may influence biodegradation of waste chemicals in surface and near-surface soils. Although TCE is stable under many environmental conditions, aerobic degradation by microorganisms has been documented for methane-treated soils (17) and sediments from TCE-contaminated aquifers (4, 7, 10), indicating that natural selection or enrichment of TCE-degrading populations can occur. Furthermore, at least some aerobic bacteria that degrade TCE appear to be methane oxidizers (7, 17), and methane oxidizers are likely to be present in soils and sediments at the capillary fringe, a zone that marks the maximum depth of deep-rooted plants. Because methane oxidizers are likely to be found in zones that fluctuate between aerobic and anerobic conditions, such as soils that periodically flood and drain (1), these bacteria may contribute to TCE degradation in the MCB. Thus, the frequency of wetting and drying may be an important factor influencing TCE degradation at the MCB. In addition, nutritional status, soil aeration, % OC, and species-specific properties of the plants (such as the ratio of root biomass to surface area, root depth, or the presence of nitrogen-fixing bacteria and mycorrhizae) may be important factors that can affect the microbial community associated with plant roots and the biodegradation of waste chemicals by this community. These findings indicate that vegetation may be an important factor influencing biological remediation of contaminated soils.

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